

Fragmentation of Singly-charged Peptide Derivatives Produced by Atmospheric Pressure Matrix-assisted Laser Desorption Ionization

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Overview

- Address difficulty in fragmenting singly-charged AP-MALDI peptides ions in ion trap [1,2]
- Use previously reported sulfonic acid derivatization process [3]
- Compare fragmentation patterns
 - ES MS/MS of doubly-charged species produce more fragments than ES MS/MS of singly-charged analyte
 - Derivatization improves fragmentation in singly-charged peptide derivatives by AP-MALDI MS/MS (and ES MS/MS)

Objective

- ▷ Use the sulfonic acid derivatization process [3] to make peptide ions formed by AP-MALDI easier to fragment into predominantly y-type ions in an ion trap

Introduction

- ☒ AP-MALDI tends to produce singly-charged ions, as does conventional MALDI
- ☒ PROBLEM: Ion trap MS/MS typically utilizes fragmentation of multiply-charged ions; higher charge state increases energy of collisions in the ion trap
- ☒ NEED: A way to better fragment AP-MALDI singly-charged ions in an ion trap
- ☒ SOLUTION: Use sulfonic acid derivatives which would allow for easier fragmentation of the singly-charged AP-MALDI peptide ions

Methods

- Derivatize Angiotensin I (ATI), Substance P (Sub P), ASHLGLAR, and cytochrome c (cyto c) tryptic digest using chlorosulfonylacetyl chloride [3]
- Analyze peptides and peptide derivatives by ES MS/MS and AP-MALDI MS/MS on a Finnigan LCQ IT-MS to determine fragmentation patterns

Sulfonic Acid Derivative

- Normal peptide

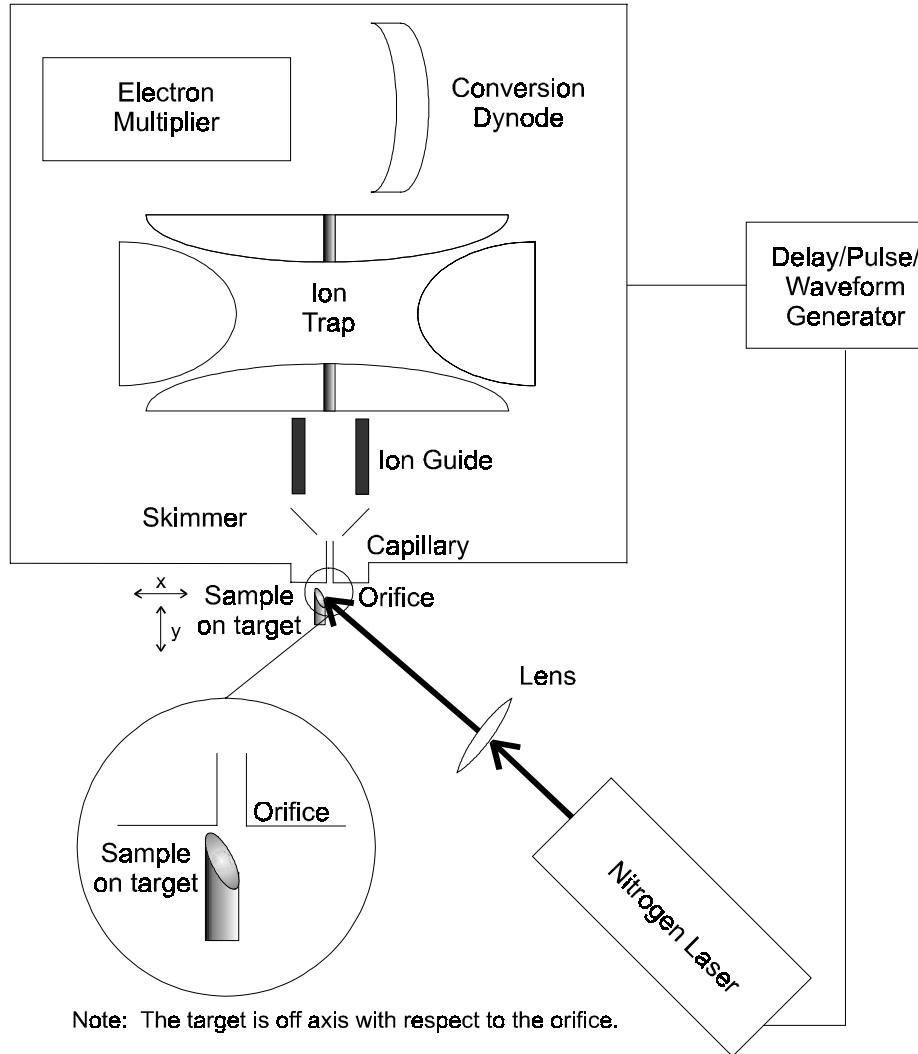
 - $\text{H}_2\text{N}-(\text{peptide sequence})-\text{CO}_2\text{H}$

- Peptide derivative

 - $-\text{O}_3\text{SCH}_2\text{CONH}-(\text{peptide sequence})-\text{CO}_2\text{H}$

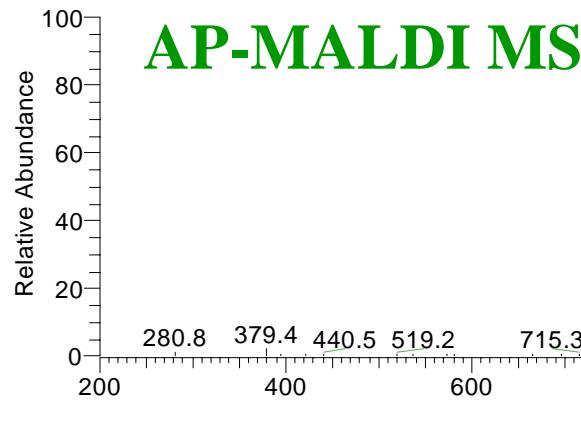
- 1) Sulfonic acid attaches to the N-terminus
- 2) First added proton (at most basic site, such as arginine side chain) produces neutral molecule
- 3) Second added proton (to produce +1 state) now has higher likelihood to localize along chain, inducing y-type fragmentation

AP-MALDI IT MS



ATI Spectral Features of AP-MALDI and ESI Differ

S#: 1-10 RT: 0.01-0.15 AV: 10 NL: 1.44E6
T: + c ms [200.00 - 2000.00]



[ATI + H]⁺

1296.7

[ATI + 2CHCA + H]⁺

1298.7

1318.6

1674.6

1676.0

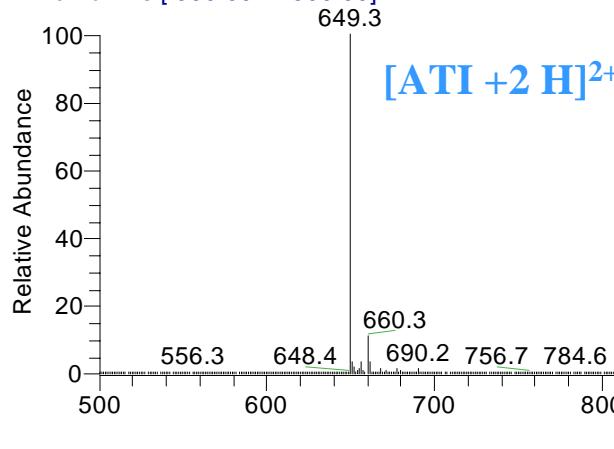
1545.7

1672.9

1845.9

1921.7

S#: 1-15 RT: 0.01-0.25 AV: 15 NL: 1.26E8
T: + c Full ms [500.00 - 1500.00]



[ATI + 2 H]²⁺

[ATI + H]⁺

1296.7

1297.7

1298.7

1318.6

1359.4

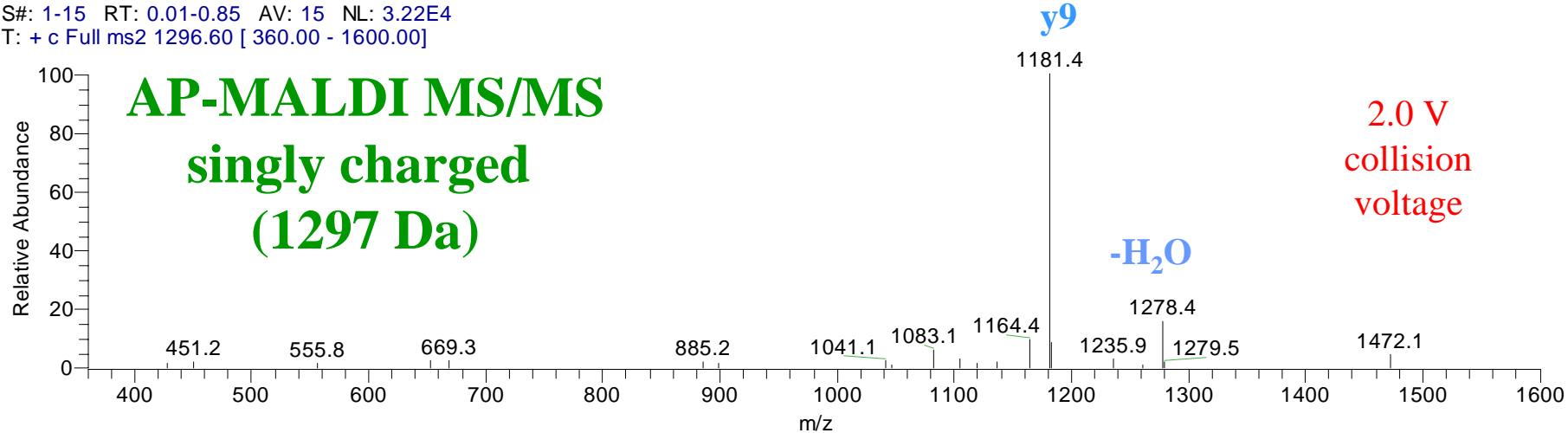
1442.4

1471.2

ATI Fragmentation Patterns Depend on Charge State

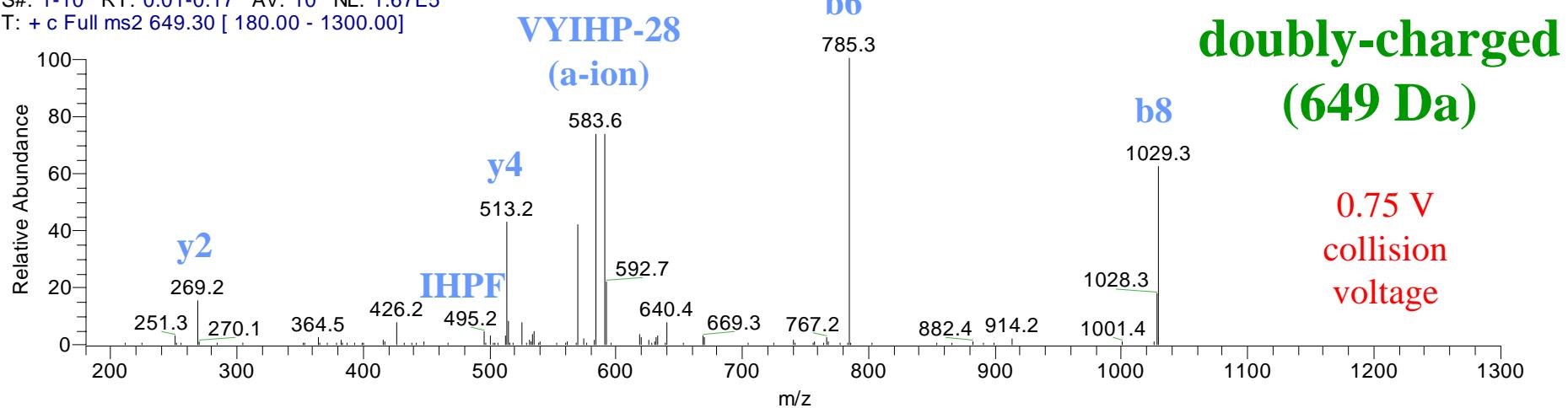
S#: 1-15 RT: 0.01-0.85 AV: 15 NL: 3.22E4
T: + c Full ms2 1296.60 [360.00 - 1600.00]

AP-MALDI MS/MS
singly charged
(1297 Da)



S#: 1-10 RT: 0.01-0.17 AV: 10 NL: 1.67E5
T: + c Full ms2 649.30 [180.00 - 1300.00]

VYIHP-28
(a-ion)

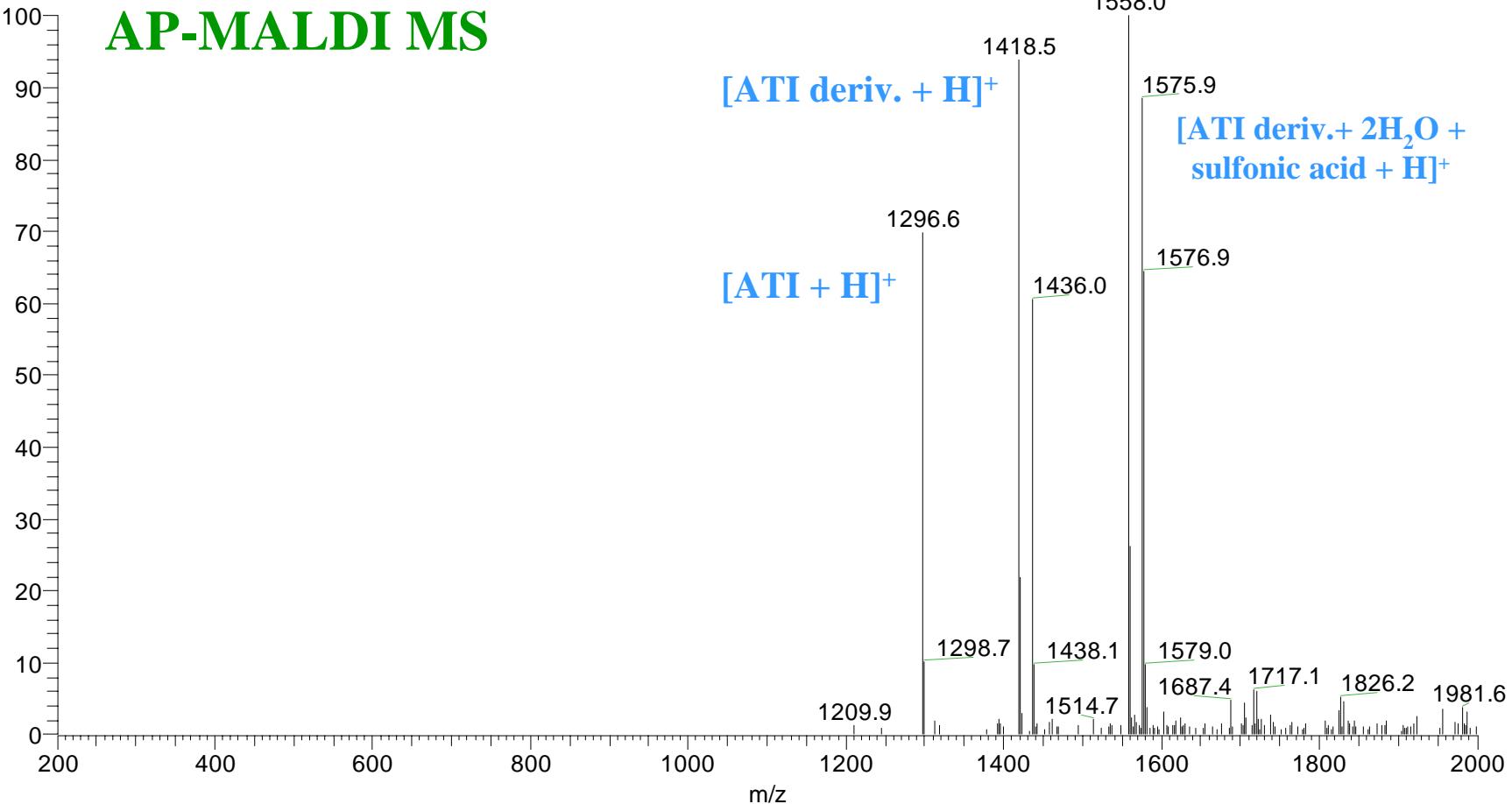


Derivatization Attaches Several Sulfonic Acid Groups

S#: 1-15 RT: 0.01-0.21 AV: 15 NL: 2.55E5
T: + c ms [200.00 - 2000.00]

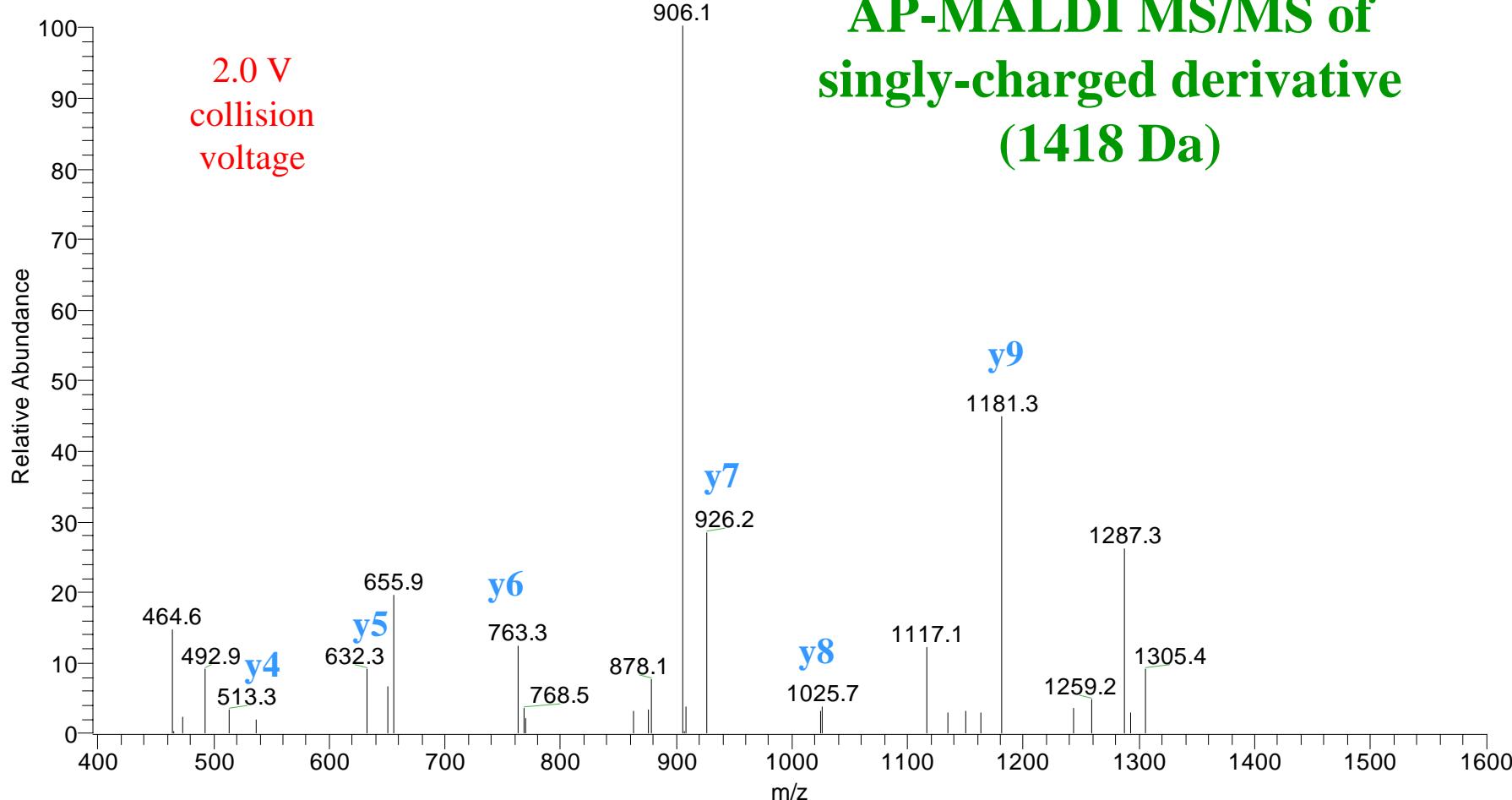
AP-MALDI MS

Relative Abundance



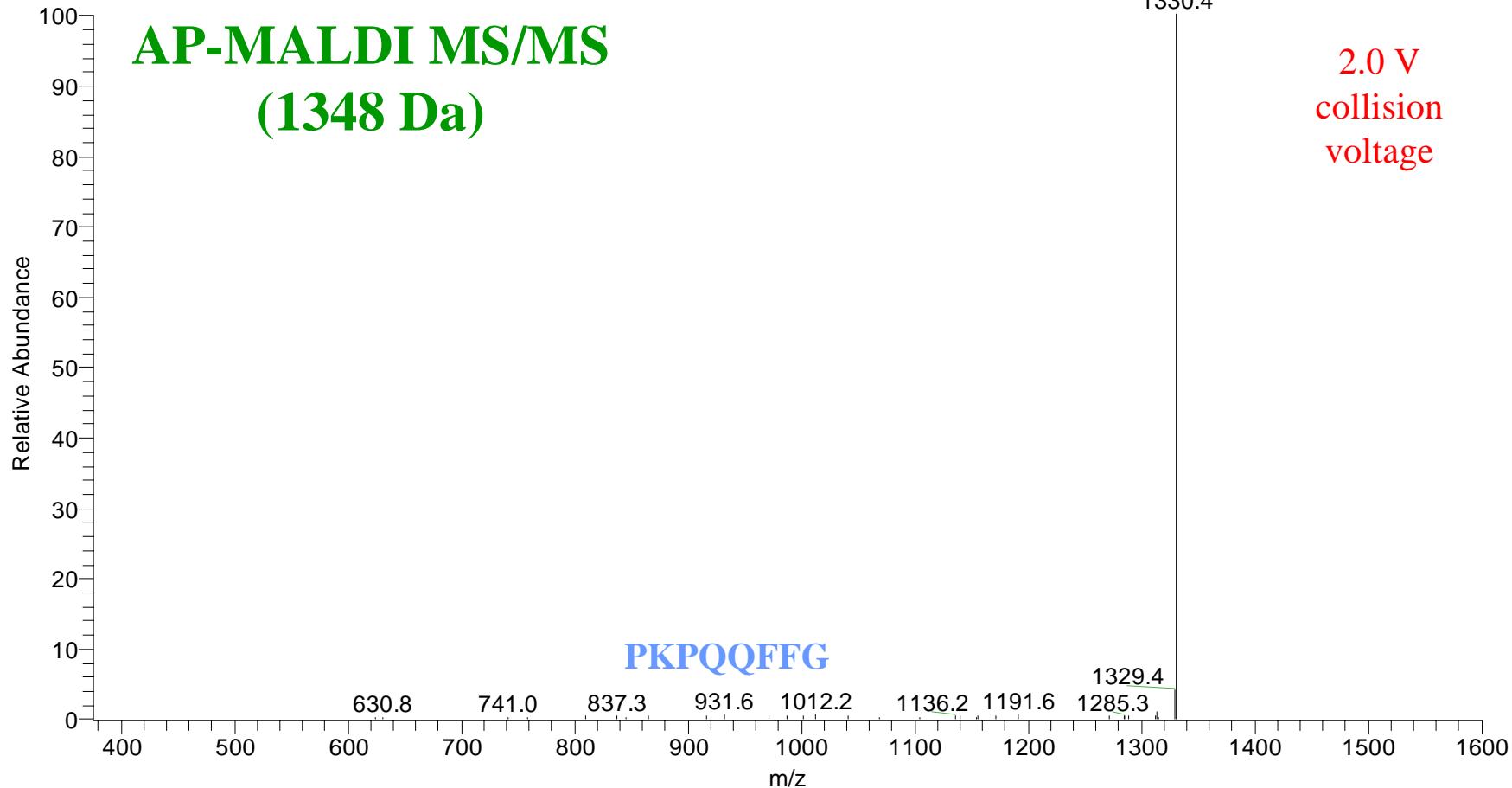
ATI Derivative Fragments into y-ions

S#: 1-15 RT: 0.02-0.85 AV: 15 NL: 1.71E4
T: + c Full ms2 1418.50 [395.00 - 1600.00]



Sub P Fragments Poorly

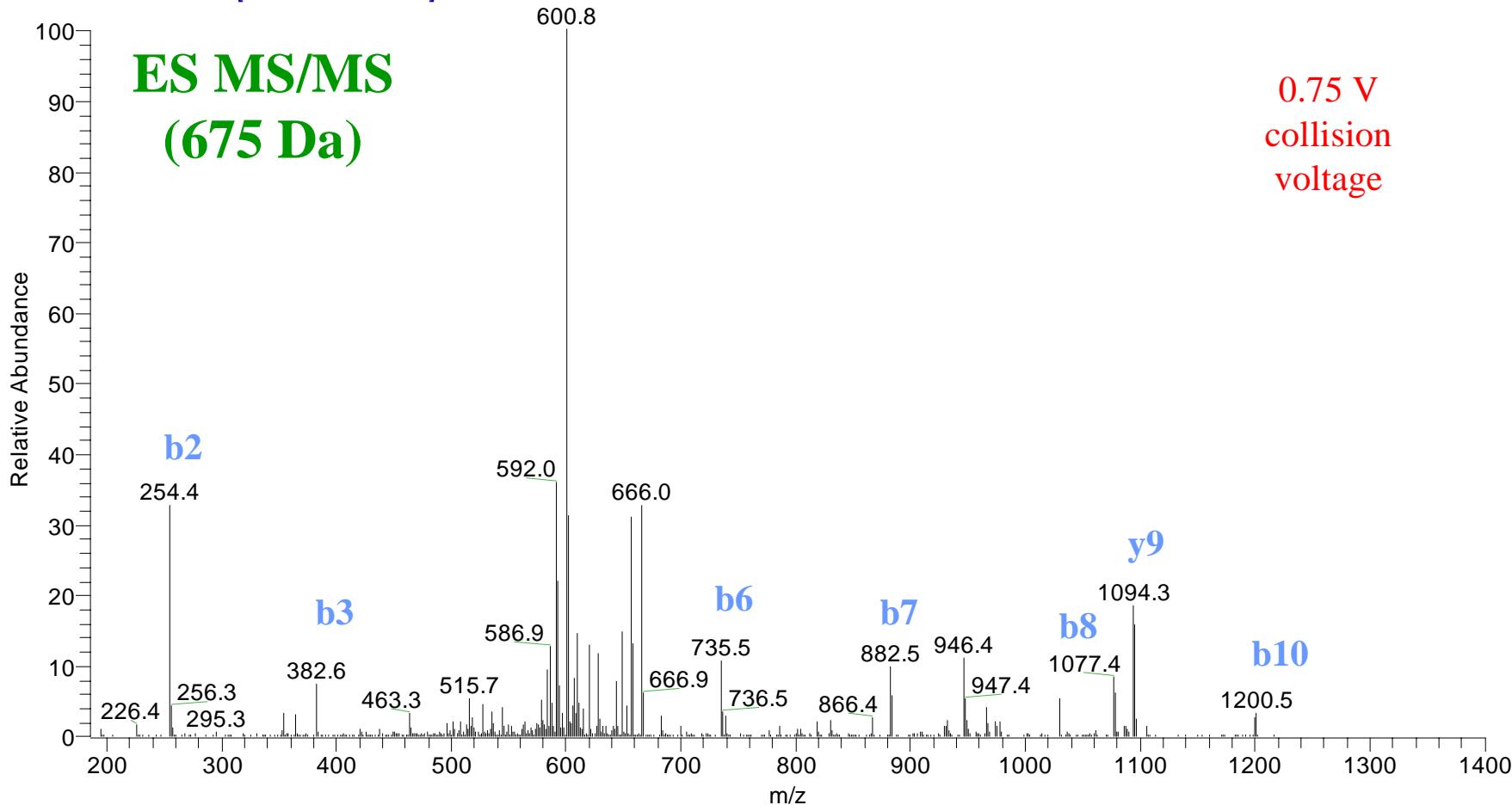
S#: 1-15 RT: 0.01-0.84 AV: 15 NL: 2.71E5
T: + c Full ms2 1348.00 [375.00 - 1600.00]



Note: amide at C-terminus on Substance P

Doubly-Charged ES Sub P Fragments Well

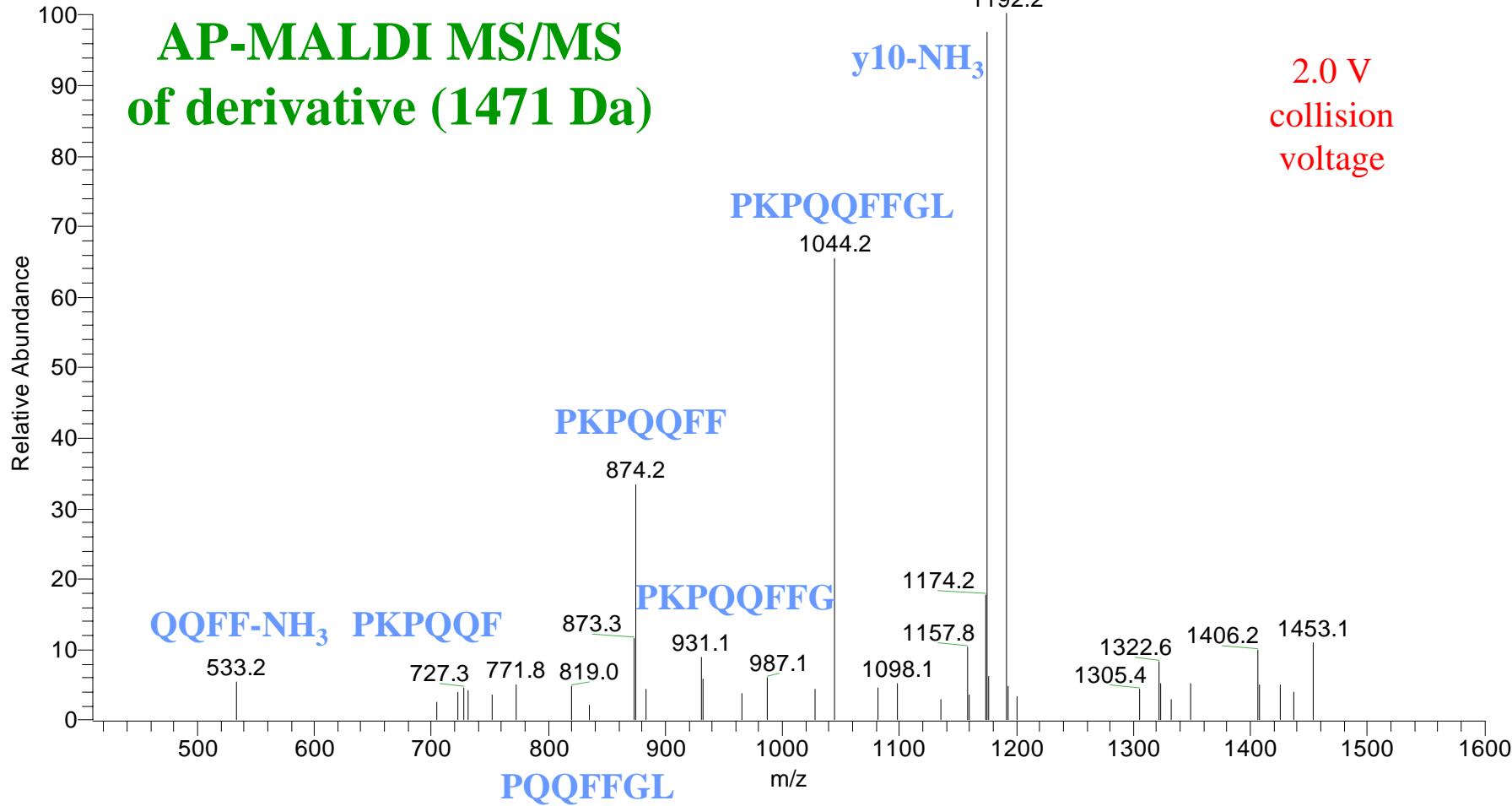
S#: 1-10 RT: 0.01-0.16 AV: 10 NL: 1.60E6
T: + c Full ms2 675.00 [185.00 - 1400.00] $[b_{10}+2H]^{2+}$



Singly-Charged Sub P Derivative Fragments Well

S#: 1-15 RT: 0.03-0.85 AV: 15 NL: 1.45E4
T: + c Full ms2 1470.90 [410.00 - 1600.00]

**AP-MALDI MS/MS
of derivative (1471 Da)**



Sub P Sulfonic Acid Derivative

- Substance P with amide C-terminus



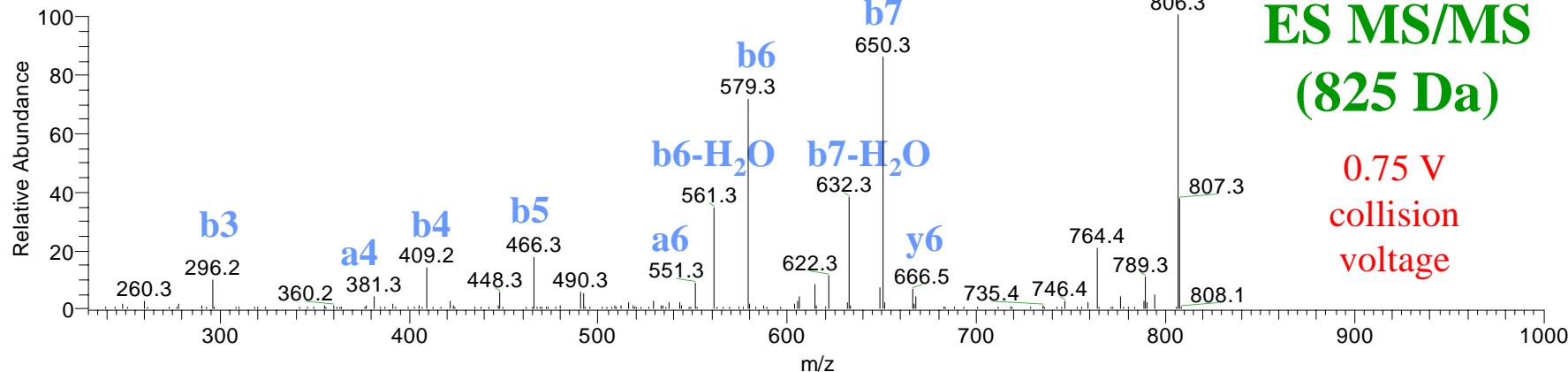
- Resulting Substance P derivative



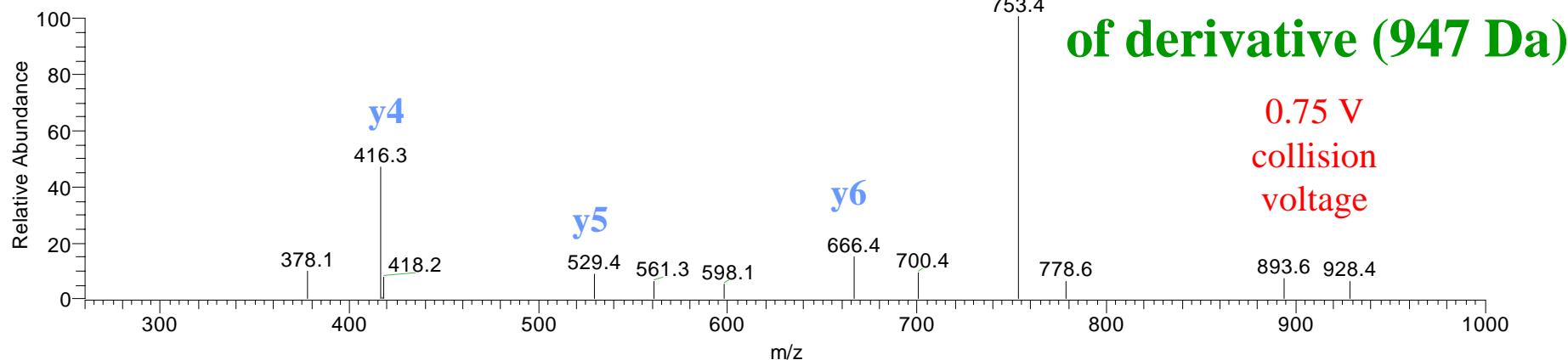
- Because of the amide, the sulfonic acid attaches to the C-terminus instead of the N-terminus.

Simpler ASHLGLAR MS/MS Spectrum from Derivative

S#: 1-10 RT: 0.02-0.21 AV: 10 NL: 7.69E4
T: + c Full ms2 824.90 [230.00 - 1000.00]



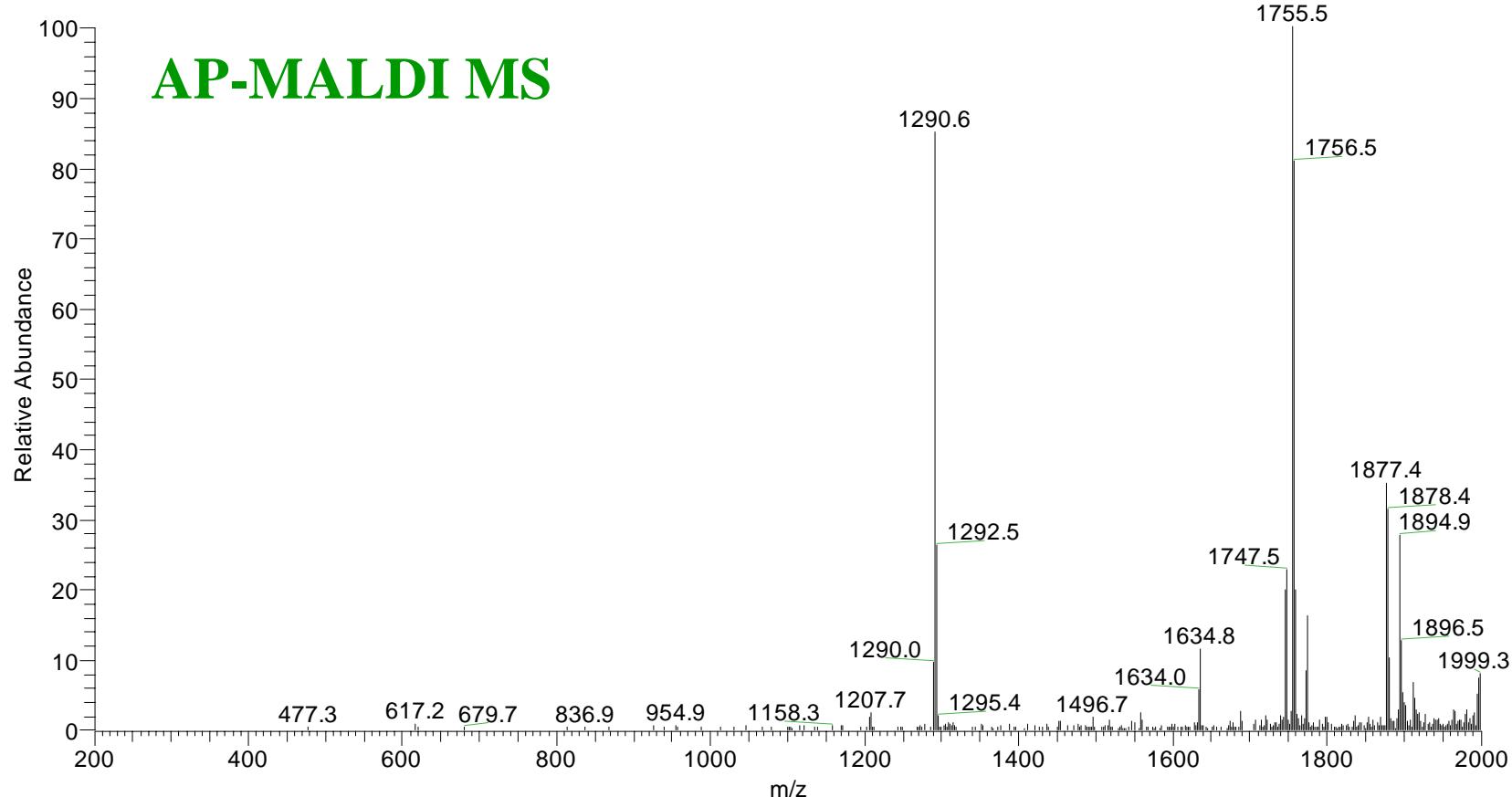
S#: 1-10 RT: 0.00-0.47 AV: 10 NL: 1.69E4
T: + c Full ms2 946.40 [260.00 - 1000.00]



Derivatization Works for Cyto C Tryptic Digest

S#: 1-15 RT: 0.00-0.21 AV: 15 NL: 7.56E5

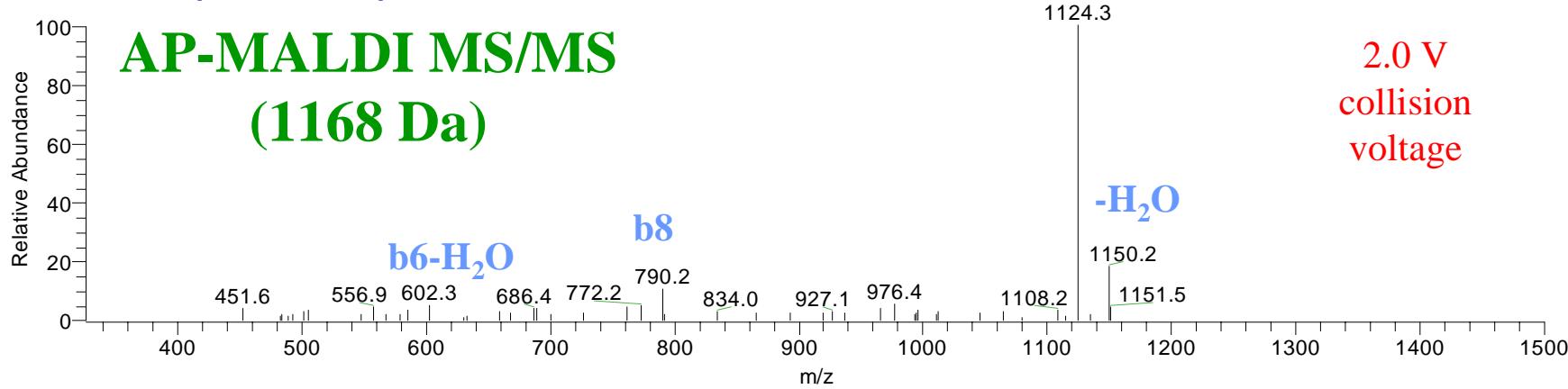
T: +c ms [200.00 - 2000.00]



Derivatization Improves MS/MS of Fragment TGPNLHGLFGR

S#: 1-14 RT: 0.03-0.79 AV: 14 NL: 2.79E4

T: + c ms2 1168.60 [325.00 - 1500.00]



Conclusions

- As expected, ES MS/MS of doubly charged species produces more peptide mapping information than AP-MALDI MS/MS of singly charged analytes
- Singly-charged peptide derivatives show fragmentation with mostly y-ions

Conclusions

- The amide C-terminus of Substance P changes the expected fragmentation scheme
- Sulfonic acid derivatives enhance the utility of AP-MALDI MS/MS for peptides

References

- (1) V.V. Laiko, M.A. Baldwin, A.L. Burlingame, *Anal. Chem.*, 2000, **72**, 652-657.
- (2) V.V. Laiko, S.C. Moyer, and R.J. Cotter, *Anal. Chem.*, 2000, **72**, 5239-5243.
- (3) T. Keough, R.S. Youngquist, and M.P. Lacey, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 7131-7136.

Acknowledgements

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